attachment (G) glycoproteins (ref. 9). The F protein is synthesized as a 68 kDa precursor molecule (FO) which is proteolytically cleaved into disulfide-linked F1 (48 kDa) and F2 (20 kDa) polypeptide fragments (ref. 10). The G

5   protein (33 kDa) is heavily O-glycosylated giving rise to a glycoprotein of apparent molecular weight of 90 kDa (ref. 11). Two broad subtypes of RS virus have been defined: A and B (ref. 12) The major antigenic differences between these subtypes are found in the G

10   glycoprotein (refs. 7, 13).

A safe and effective RS virus vaccine is not available and is urgently needed. Approaches to the development of RS virus vaccines have included inactivation of the virus with formaldehyde, isolation of

15   cold-adapted and/or temperature-sensitive mutant viruses and isolation of the protective antigens of the virus. Clinical trial results have shown that both live attenuated and formalin-inactivated vaccines failed to adequately protect vaccinees against RS virus infection

20   (refs. 14 to 16). Problems encountered with cold-adapted and/or temperature-sensitive RS virus mutants administered intranasally included clinical morbidity, genetic instability and overattenuation (refs. 17 to 19). A live RS virus vaccine administered subcutaneously also

25   was not efficacious (ref. 20). Inactivated RS viral vaccines have typically been prepared using formaldehyde as the inactivating agent. Murphy et al. (ref. 21) has reported data on the immune response in infants and children immunized with formalin-inactivated RS virus.

30   Infants (2 to 6 months of age) developed a high titre of antibodies to the F glycoprotein but had a poor response to the G protein. Older individuals (7 to 40 months of age) developed titres of F and G antibodies comparable to those in children who were infected with RS virus.

35   However, both infants and children developed a lower level of neutralizing antibodies than did individuals of

comparable age with natural RS virus infections. The unbalanced immune response, with high titres of antibodies to the main immunogenic RS virus proteins F (fusion) and G (attachment) proteins but a low
5 neutralizing antibody titre, may be in part due to alterations of important epitopes in the F and G glycoproteins by the formalin treatment. Furthermore, some infants who received the formalin-inactivated RS virus vaccine developed a more serious lower respiratory
10 tract disease following subsequent exposure to natural RS virus than did non-immunized individuals (refs. 15, 16). The formalin-inactivated RS virus vaccines, therefore, have been deemed unacceptable for human use.

Evidence of an aberrant immune response also was
15 seen in cotton rats immunized with formalin-inactivated RS virus (ref. 22). Furthermore, evaluation of RS virus formalin-inactivated vaccine in cotton rats also showed that upon live virus challenge, immunized animals developed enhanced pulmonary histopathology (ref. 23).

20 The mechanism of disease potentiation caused by formalin-inactivated RS virus vaccine preparations remains to be defined but is a major obstacle in the development of an effective RS virus vaccine. The potentiation may be partly due to the action of formalin
25 on the F and G glycoproteins. Additionally, a non-RS virus specific mechanism of disease potentiation has been suggested, in which an immunological response to contaminating cellular or serum components present in the vaccine preparation could contribute, in part, to the
30 exacerbated disease (ref. 24). Indeed, mice and cotton rats vaccinated with a lysate of HEp-2 cells and challenged with RS virus grown on HEp-2 cells developed a heightened pulmonary inflammatory response.

Furthermore, RS virus glycoproteins purified by
35 immunoaffinity chromatography using elution at acid pH were immunogenic and protective but also induced

immunopotentiation in cotton rats (refs. 22, 25).

There clearly remains a need for immunogenic preparations, including vaccines which are not only effective in conferring protection against disease caused by RS virus but also does not produce unwanted side-effects, such as immunopotentiation. There is also a need for antigens for diagnosing RSV infection and immunogens for the generation of antibodies (including monoclonal antibodies) that specifically recognize RSV proteins for use, for example, in diagnosis of disease caused by RS virus.

Art recognized approaches to the developments of RSV vaccines have been summarized in recent review articles (refs. 2, 31 to 35), none of which propose the development of an inactivated RSV vaccine.

SUMMARY OF THE INVENTION

The present invention provides a novel approach to the provision of such antigens and immunogens by inactivation of purified RS virus.

In one aspect of the present invention, there is provided a method of preparing an immunogenic composition capable of producing a respiratory syncytial (RS) virus specific immune response in a host immunized therewith, particularly a human host, which comprises a plurality of steps. The RS virus first is grown on an appropriate cell line and the virus harvested. The harvested virus is purified under non-denaturing conditions to produce a purified virus substantially free from cellular and serum components. The purified virus then is inactivated with an inactivating agent to provide a non-infectious, non-immunopotentiating and immunogenic RS virus. This RS virus then is formulated as an immunogenic composition.

The inactivating agent may be β-propiolactone; a non-ionic detergent, including n-octyl-α-D glucopyranoside and n-octyl-β-D-glucopyranoside; or ascorbic acid.

The purifying step which is carried out on the harvested virus preferably may be effected by microfiltration to remove cell debris, tangential flow ultrafiltration to remove serum components, particularly employing an about 100 to about 300 kDa nominal molecular weight cut-off membrane, pelleting the ultrafiltered material by ultracentrifugation to further remove serum components and subjecting the pelleted material to sucrose density gradient centrifugation. Alternatively, the retentate from tangential flow ultrafiltration may be subjected to gel filtration followed by ion-exchange chromatography to further remove serum components.

This procedure provides a novel immunogenic composition capable of producing an RS virus specific immune response in a host immunized therewith which constitutes a further aspect of the present invention. Such immunogenic composition comprises purified, inactivated RS virus which is substantially free from cellular and serum components and which is non-infectious, non-immunopotentiating, immunogenic and protective, and a carrier therefor. The immunogenic composition may be formulated as a vaccine for *in vivo* administration to a human host for protecting the human from a disease induced by RS virus. The carrier for the immunogenic composition may comprise an adjuvant. The immunogenic composition may be formulated as a vaccine to be administered in an injectable form, intranasally or orally.

The present invention further provides a method of immunizing a host, particularly a human host, against disease caused by RS virus, which comprises administering to the host an effective amount of the immunogenic composition provided herein. The host immunized by such procedure may be selected from infants, young children, pregnant women, women of childbearing age, elderly individuals, immunocompromised individuals and other

virus adsorption, the infected cells are washed in culture medium and virus growth is monitored for 5 to 7 days post-infection.

Virus Processing and Concentration

5   The harvested virus then is purified under non-denaturing conditions to be substantially free from cellular and serum components. Such purification may be effected in any convenient manner.   In one such procedure, the RS virus supernatant is microfiltered

10   (0.22 to 8 μm pore size filters) to remove cell debris. The clarified viral fluid then may be concentrated by tangential flow ultrafiltration using an ultrafiltration membrane with a molecular weight cut-off between about 100 to about 300 kDa, to remove serum components.

15   The concentrated RS virus is subjected to further purification for utilization in the immunogenic preparations and as antigens.   In one procedure, the concentrated virus may be pelleted by ultracentrifugation and the supernatant from the ultracentrifugation is

20   discarded thereby further removing serum components. The pelleted virus is resuspended in PBS or another suitable medium.   The concentrated virus then is purified by sucrose density gradient ultracentrifugation. Alternatively, the retentate from the tangential flow

25   ultrafiltration step may be subjected to gel filtration followed by ion-exchange chromatography to further remove serum components. The resulting RS viral material may be further pelleted by ultracentrifugation.   The pelleted-purified RS virus may be resuspended in PBS and stored at

30   -70°C pending use.

Virus Inactivation

The purified RS virus next is inactivated. Such inactivation is effected using materials which provide the purified virus in a non-infectious, non-

35   immunopotentiating and immunogenic form.   The inactivating agent employed in this step generally

comprises β-propiolactone, ascorbic acid or a non-ionic detergent. Among the non-ionic detergents which may be employed in the inactivation step are certain glucopyranosides, including n-octyl-β-D-glucopyranoside and n-octyl-α-D-glucopyranoside.

Any convenient quantity of inactivating agent and any desired reaction conditions may be employed consistent with the desire to provide a non-infectious, non-immunopotentiating and immunogenic material.

As an example, RS virus may be inactivated using about a 0.1% solution of β-propiolactone (BPL) for about 30 to 120 minutes or ascorbic acid for about 24 hours at about 37°C with constant shaking. The residual BPL may be removed from the inactivated sample by dialysis against PBS using a 10,000 to 20,000 molecular weight membrane.

Immunogenicity Studies in Cotton Rats

The purified and inactivated RS viral material provided by this procedure is immunogenic and protective while being non-infectious. This result was determined by evaluation of the immunogenicity RS viral materials in cotton rats including their capacity to protect these animals from live RS virus challenge, as reported below. Vaccine preparations provided herein elicited RS virus specific neutralizing antibodies and protected cotton rats from live virus challenge.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis, treatment of diseases caused by respiratory syncytial virus and the generation of immunological reagents. A further non-limiting discussion of such uses is further presented below.

Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from inactivated RSV as

REFERENCES:

1. Glezen, W.P., Paredes, A. Allison, J.E., Taber, L.H. and Frank, A.L. (1981). J. Pediatr. 98, 708-715.

2. Chanock, R.M., Parrot, R.H., Connors, M., Collins, P.L. and Murphy, B.R. (1992) Pediatrics 90, 137-142.

3. Martin, A. J., Gardiner, P.S. and McQuillin, J. (1978). Lancet ii, 1035-1038.

4. Robbins, A., and Freeman, P. (1988) Sci. Am. 259, 126-133.

5. Glezen, W.P., Taber, L.H., Frank, A.L. and Kasel, J.A. (1986) Am. J. Dis. Child. 140, 143-146.

6. Katz, S.L. New vaccine development establishing priorities. Vol. 1. Washington: National Academic Press. (1985) pp. 397-409.

7. Wertz, G.W., Sullender, W.M. (1992) Biotech. 20, 151-176.

8. McIntosh, K. and Chanock, R.M. (1990) in Fields Virology (Fields, B.M., and Knipe, D.M. eds.) pp. 1045-1075, Raven Press, Ltd., New York.

9. Walsh, E.E., Hall, C.B., Briselli, M., Brandiss, M.W. and Schlesinger, J.J. (1987) J. Infect. Dis. 155, 1198-1204.

10. Walsh, E.E., Hruska, J. (1983) J. Virol. 47, 171 177.

11. Levine, S., Kleiber-France, R., and Paradiso, P.R. (1987) J. Gen. Virol. 69, 2521-2524.

12. Anderson, L.J., Hierholzer, J.C., Tsou, C., Hendry, R.M., Fernie, B.F., Stone, Y. and McIntosh, K. (1985),J. Infect. Dis. 151, 626-633.

13. Johnson, P.R., Olmsted, R.A., Prince, G.A., Murphy, B.R., Alling, D.W., Walsh, E.E. and Collins, P.L. (1987) J. Virol. 61 (10), 3163-3166.

14 Fulginiti, V.A., Eller, J.J., Sieber, O.F., Joyner, i.W., Minamitani, M. and Meiklejohn, G. (1969) Am. J. Epidemiol. 89 (4), 435-448.

15. Chin, J., Magoffin, R.L., Shearer, L.A., Schieble, J.H. and Lennette, E.H. (1969) Am. J. Epidemiol. 89 (4), 449-463.

16. Kapikian, A.Z., Mitchell, R.H., Chanock, R.M., Shvedoff, R.A. and Stewart, C.E. (1 9 6 9) Am. J. Epidemiol. 89 (4), 405-421.

17. Kim, H.W., Arrobio, J.O., Pyles, G., Brandt, C.D Camargo, E., Chanock, R.M. and Parrott, R.H. (1971 Pediatrics 48, 745-755.

18. Wright, P.F., Belshe, R.B., Kim, H.W., Van Voris, L.P. and Chanock, R.M. (1982) Infect. Immun. 37 (1), 397-400.

19. Wright, P.F., Chinozaki, T. and Fleet, W. (1976) J. Pediatr. 88, 931-936.

20. Belshe, R.B., Van Voris, L.P. and Mufson, M.A. (1982) J. Infect. Dis. 145, 311-319.

21. Murphy, BR., Prince, G.A., Walsh, E.E, Kim H.W., Parrott, R.H., Hemming V.G., Rodriguez, W.J., and Chanock. J. Clin. Microbiol. (1986), 24 (2), 197-202.

22. Connors, M., Collins, P.L., Firestone, C-Y., Sotnikov, A.V., Waitze, A., Davis, A.R., Hung, P.P., Chanock, R.M., Murphy, B. (1992) Vaccine, 10, 475-484.

23. Prince, G.A., Jenson, A.B., Hemming, V.G., Murphy, E.R., Walsh, E.E., Horswood, R.L and Chanock, R.L. (1986 b) J. Virol. 57 (3), 721-728.

24. Piedra, P.A., Camussi, F. and Ogra, P.L. (1989) J. Gen. Virol. 70, 325-333.

25. Walsh, E.E., Hall, C.B., Briselli, M., Brandiss, M.W. and Schlesinger, J.J. (1987) J. Infect. Dis. 155 (6), 1198-1204.

26. Budowsky, E.I. and Zheleznova, N.V. (1991) Vaccine 9 (6), 319-325.

27. Budowsky, E.I., Friedman, E.A., Zheleznova, N.V. and Noskov, F.S. (1991) Vaccine 9 (6), 398-402.

28. Stephan, W., Dichtelmuller, H., Prince, A.M., Brotman, B. and Huima, T. J. Med. Virol. (1988), 26 (3), 227-232.

29. Barth, R., Gruschkau, H., Bijok, U., Hilfenhaus, J., Hinz, J. and Milcke, L. J. Biol. Stand. (1984), 12 (1), 29-46.

30. Salkind, A.R. and Roberts N.J. (1992) Vaccine. 10, 519-523.

31. Murphy, B.R., Prince, G.A., Collins, P.L. Coelingh, K.V.W., Olmsted R.A., Spriggs, M.K., Parrott, R.H., Kim, H-W., Brandt, C.D. and Chanock, R.M. (1988) Virus Research 11, 1-15.

32. Kimman, T.G. and Westenbrink, F. (1990) Archives of Virology 112, 1-25.

33. Wertz, G.W. and Sullender, W.M. (1992) Biotechnology 20: 151-176.

34. La Via, W.V., Marks, M.I., Stutman H.R. (1992) J. Pediatrics 121, 503-510.